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Antibacterial and antibiotic-modulating activities of *Rhinella jimi* and three other animal extracts against multidrug-resistant Gram-negative phenotypes

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Abstract

Background: The antibiotic resistance of pathogenic bacteria is one of the major health problems worldwide. The development of novel antimicrobial therapies based on natural products that greatly reduce this resistance is urgent. The present study aimed at evaluating the antimicrobial potential of four animal methanol extracts, *Gryllus campestris*, *Testudo hermanni*, *Cardisoma guanhumi,* and *Rhinella jimi* as well as their synergistic effects with antibiotics against twenty Gram-negative bacteria.

Methods: Zoochemical analysis of extracts was performed using qualitative reference methods for the detection of secondary metabolites and the ninhydrin reaction for the detection of protein constituents. The antibacterial activity of animal extracts alone and in combination with antibiotics was carried out using broth microdilution methods.

Results: Amino acids, peptides, or proteins were present in all extracts. Alkaloids were detected in extracts of *C. guanhumi* and *R. jimi* and were absent in other extracts. Flavonoids, tannins, and steroids were evidenced only in dried and fresh extracts of *R. jimi*. Polyphenols, anthocyanins, anthraquinones, and saponins were not detected in all extracts. Dried extract from *R. jimi* was most active. It had antibacterial potential against 85% of the tested bacterial strains with significant activity (100≤MIC≤512 µg/mL) against 35% of bacteria; this included three *E. coli* (ATCC8739, AG100A$_{Tet}$ and MC4100), one *E. aerogenes* (ATCC13048), one *K. pneumoniae* (ATCC11296) and two *P. aeruginosa* (PA01 and PA124). Dried and fresh extracts from *C. guanhumi* displayed an antibacterial activity against 40% and 20% of the bacteria tested, respectively, whereas dried extracts from *G. campestris* and fresh extracts from *T. hermanni* inhibited the growth of 15% and 10% of bacteria, respectively (MIC range of 512 to 2048 µg/mL). The dried and fresh extracts of *R. jimi*, at MIC/2 and MIC/4, potentialized the activities of more than 70% of the antibiotics respectively against more than 70% of studied bacteria. Both extracts highly improved the activity of oxacillin, gentamicin, erythromycin, and ciprofloxacin with improved activity factors (IAFs) ranging from 16 to 256.

Conclusion: This work demonstrated that *R. jimi* extracts had a broad spectrum of antibacterial activities. The overall data provided evidence that animals investigated in this study might be potential sources of natural antimicrobial agents; They can be combined with clinically used antibiotics to overcome bacterial resistance.

Keywords: Animal extract, antibiotics; Gram-negative bacteria; efflux pumps; multidrug resistance; *Rhinella jimi.*

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Background

Despite the considerable progress in medicine, the treatment of infectious diseases continues to face many challenges. The occurrence of severe levels of antibiotic resistance is the main issue facing medical practitioners [1,2]. Multidrug resistance (MDR) in bacteria is usually mediated by the expression of efflux pumps or porins involved in transport, by the expression of mutated genes coding for specific drug targets or specific enzymatic barriers. As a matter of fact, MDR remains a major obstacle hindering successful antibacterial chemotherapy [3-5]. Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat. Unfortunately, resistance has eventually been seen to nearly all antibiotics that have been developed [6]. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria. It is an increasingly serious threat with a global public health impact that requires action across all government sectors and society. Resistant bacteria cause severe clinical diseases for a longer period leading to a huge economic burden. Documented data revealed that pathogenic bacteria and especially *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi,* and *enterobacteria* had high levels of resistance against many classes of antibiotics [7,8]. Bacterial infections are among the ten causes of death worldwide according to the World Health Organisation [9,10]. The presence and emergence of resistance strains make the risk of infections a universal problem with deleterious effects. Therefore, the incidence of resistance to existing antibiotics by microorganisms demands increased effort in the development of new antibiotics for the treatment of microbial infections and diseases. The discovery of new alternatives is necessary for the treatment of infections involving resistant microorganisms. Natural products from plants including botanicals and phytochemicals have been intensively investigated during the three last decades, for their potential against various human diseases, and mostly bacterial infections, cancer, and other inflammatory disease [11-29]. Recently, new antimicrobial peptides from natural sources have drawn attention as antimicrobial agents. Since antimicrobial peptides were initially identified in frogs and insects in the 1980s, many additional peptides have been found and over 1200 of them have been isolated from animals to date [30,31]. The first antimicrobial peptide, developed in the 1990s, is a magainin, pexiganan, which is a widely used anionic antimicrobial peptide. In a clinical study, this peptide, incorporated into a cream, proved to be as effective as oral antibiotic therapy with ofloxacine, in the treatment of superficial skin ulcers in diabetic patients [32]. Although invertebrates have recently been shown to be capable of some form of specific immune memory [33], their primary mode of enhancing future resistance is probably through systemic upregulation of relatively unspecific immune components [34]. As the incidence of MDR strains continues to increase, treatment options become very limited and there is an urgent need for rational combination therapy with maximum killing and minimal emergence of resistance. Repurposing veterinary medicines for human use has gained significant interest recently, and several potential combinations have been identified [35,36]. Herein, we report the antibacterial activities of the methanol extracts of four animals from Cameroon including *Gryllus campestris* Linnaeus (Gryllidae), *Cardisoma guanhumi* Latreille (Gecarcinidae), *Testudo hermanni* Mojsisovics (Testudinidae), and *Rhinella jimi* Stevaux (Bufonidae), as well as their synergistic effects with some commonly used antibiotics.

Methods

Collection of test samples

Four Cameroonian vertebrates and a commonly comestible animal were used. They were composed of *Gryllus campestris*, *Testudo hermanni*, *Cardisoma guanhumi* and *Rhinella jimi* and they were collected in the Littoral and West regions of Cameroon in October 2020. They were then identified at the laboratory of animal biology of the University of Dschang, Cameroon, where each specimen was deposited.

Media for bacterial cultivation and microorganisms

Two culture media were used for bacterial cultivation. Mueller Hinton agar (MHA) was used for the activation of bacteria strains and Mueller Hinton broth (MHB) was used for the determination of minimal inhibitory and bactericidal concentrations. Twenty bacterial strains constituted of drug-sensitive and multidrug.resistant (MDR) Gram-negative strains expressing efflux pumps were used; they included reference strains and clinical isolates of *Escherichia coli* (ATCC8739, AG100A, AG102, AG100Atet, MC4100, W3110), *Enterobacter aerogenes* (ATCC13048, EA27, EA289, EA294, EA298, CM64), *Klebsiella pneumonia* (ATCC11296, KP55, KP63), *Pseudomonas aeruginosa* (PA01, PA124), and *Providencia stuartii* (ATCC29916, PS2636, NEA16). They were provided by the American Type Culture Collection (ATCC) and by the Laboratory of UMR-MD1 of the University of Mediterranean, Marseille, France. They were maintained on an agar slant at 4°C and cultured on a fresh appropriate agar plate for 24 h prior to any antimicrobial test.

Antibiotics and other chemicals for antimicrobial assay

Nine commonly used antibiotics belonging to different families were used. They included Oxaciclin (OXA), Thiamphenicol (THI), Gentamycin (GEN), Erythromycin (ERY), Ciprofloxacin (CIP), Doxycycline (DOX), Flucloxacillin (FLC), Ofloxacin (OFL), and Azithromycin (AZT). p-Iodonitrotetrazolium chloride (INT) was used for colorimetric detection of living bacteria while dimethylsulfoxide (DMSO) was used to dissolve the extracts and antibiotics. All these substances were provided by Sigma-Aldrich (St. Quentin Fallavier, France).

Animal extraction

Animals were firstly washed with water, then flesh was isolated from shells, scales, and internal organs such as guts, pancreas, lungs, heart, and other parts different from the flesh. The fleshes were then washed with a phosphate buffer, pH 7.2 to avoid loss of proteins and water, and were dried at room temperature sheltered from the sun. Dried samples were crushed, and the obtained powders were macerated in methanol solvent in the proportions 1/3 m/v for 48 hrs shaking three times per day. After filtration using Whatman n°1 filter paper, the filtrates obtained were concentrated under reduced pressure (at 65°C) in a rotary evaporator to give the crude extracts which were dried at room temperature for complete evaporation of the solvent. These crude extracts were kept at 4°C until further use. The extractive yield (EY) in percentage (Table 1) of each sample was calculated using the following formula: EY= (crude extract weight/powder weight) x100.

Zoochemical screening

The peptides detection in crude extracts was carried out using the ninhydrin reaction [37]. Thus, in the presence of excess hot ninhydrin, amino acids, peptide, or proteins undergo oxidative deamination and decarboxylation. Ammonia condenses with two molecules of ninhydrin to form a purple complex. The detection of the main classes of the secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenes, anthraquinones, and anthocyanins (Table 3) was carried out using previously described methods [38].

Bacterial susceptibility essays

The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBC) were determined using the microdilution method as described previously [39] with some modifications. In a 96-wells plate containing 100 µl of MHB culture medium, 100 µl of animal extracts dissolved in DMSO 2.5 % was added to the first wells and then serially distributed to the other wells. Then 100 µl of bacterial suspension (2x106 UFC/ml) was added to all wells to afford 200 µl. DMSO 2.5 % and Ciprofloxacin, the reference antibiotic tested at 256 µg/mL final concentration, were respectively used as negative and positive controls. Plates were then covered and incubated at 37°C for 18 hrs after which 40 µl of INT 0.2 % were introduced and plates were reincubated at 37°C for 30 min. The INT (yellow colour) is reduced by viable bacteria to yield a pink colour. The minimal inhibitory concentration (MIC) was defined as the lowest concentration that prevented the change of this colour and which resulted in the complete inhibition of bacterial growth. Moreover, for the determination of the minimal bactericidal concentrations (MBCs), the new 96-well plates containing 150 µL of MHB, in which were added 50 µL of aliquots from the wells corresponding to MIC as well as upper concentrations were used. The microplates were incubated at 37 °C for 48 h and revelation was done as mentioned above and the lowest concentration indicating the absence of bacterial growth was considered as MBC. Each of the experiments was carried out in triplicate and at two independent times (Table 4).
In fact, the animal extract was considered to have strong activity if MIC<100 µg/mL, significant activity if 100≤MIC≤512 µg/mL, moderate activity if 512<MIC≤2048 µg/mL, and weak activity if MIC>2048 µg/mL. Moreover, the animal extract was considered to have a bactericidal effect if MBC/MIC≤4 and a bacteriostatic effect if MBC/MIC>4 [40].

Antibiotic-modulating assay

To evaluate the antibiotic resistance modifying activity of the extracts, the MIC of the antibiotic was determined in the presence or absence of the animal extracts. This was done according to the method described by [41]. After serially diluted antibiotic solutions with concentrations varying from 0.5 to 256 µg/mL, 50 µl of extract solution followed by 50 µl of bacterial inoculum (4x106 UFC/ml) were then added, and the microplates were coved and incubated at 37°C for 18h. MICs of the combination extract-antibiotics were determined by introducing 40 µl of INT 0.2 % as described above. Preliminary tests were performed against *Pseudomonas aeruginosa* PA124 strain which was the most resistant bacteria and extracts were tested at MCI/2, MIC/4, MIC/8, and MIC/16 (Data not shown). From the obtained results, two concentrations of extracts (MCI/2 and MIC/4) were chosen to be tested against the other studied bacteria. The effects of combinations were estimated by calculating the improvement activity factors (IAF) of each combination using the following formulation: MIC of antibiotic alone / MIC of combination (Tables 5-10). Each assay was also performed in triplicate and two independent times. Extract and antibiotics were considered to have synergistic, indifferent, or antagonistic effects if IAF≥2, IAF=1, or IAF≤0.5 respectively [42].

Results

Zoochemical composition of extracts

Extracts were submitted to chemical screening to detect bioactive substances responsible for the antibacterial activity. It was shown that *Gryllus campestris* and *Testudo hermanni* extracts contained protein constituents, amino acids, or peptides. These substances as well as alkaloids were detected in dried and fresh extracts of *Cardisoma guanhumi* . Moreover, five types of bioactive compounds including alkaloids, flavonoids, tannins, steroids, and proteins constituents were contained in dried and fresh extracts of *Rhinella jimi*. However, polyphenols, anthocyanins, anthraquinones, and saponins were not detected in all extracts.

Antibacterial activity of animal extracts and ciprofloxacin

The activity of tested extracts and reference antibiotic, ciprofloxacin, was done by determining their MICs and MBCs (Table 4). Results presented in this table indicate that each extract showed antibacterial activity against at least two strains. Extracts from *Rhinella jimi* were most active. Dried extract of this animal inhibited the growth of 85% of bacteria with significant activity (100≤MIC≤512 µg/mL) against 35% of bacteria including all species except *Providencia stuartii* strains. These bacteria are three *Escherichia coli* strains (ATCC8739, AG100$A_{Tet,}$ and MC4100), one *Enterobacter aerogenes* strain (ATCC13048), one *Klebsiella pneumoniae* strain (ATCC11296), and two *Pseudomonas aeruginosa* trains (PA01 and PA124). Moreover, this extract showed bactericidal effects (MBC/MIC≤4) against the same bacteria. This extract therefore showed moderate activity (512<MIC≤2048 µg/mL) against the other bacteria (50%) and was not active on three bacterial strains such as *E. coli* AG100A and *E. aerogenes* (EA289 and EA298). Fresh extract of this animal exhibited an antibacterial potential against 55% of studied strains with significant activity and bactericidal effects against two bacteria which are *E. coli* ATCC8739 and *E. aerogenes* CM64. These extracts were followed by those of *Cardisoma guanhumi* whose dried and fresh extracts inhibited the growth of 40% and 20% of bacteria respectively with moderate activity. Dried one showed activity against all *K. pneumoniae* strains and at least one strain of other species while fresh one does not inhibit the growth of all strains of *K. pneumoniae*, *P. stuartii* and *P. aeruginosa* and showed antibacterial activity against three *E. coli* strains (ATCC8739, AG100$A_{Tet,}$ and MC4100) and one *E. aerogenes* strain, CM64. Extracts from *Gryllus campestris* and *Testudo hermanni* were less active as they showed antibacterial activity only against 15% and 10% of studied strains respectively. The three bacterial inhibited by the extract from *G. campestris* are all *E. coli* strains (ATCC8739, AG102, and W3110) while those inhibited by extract of *T. hermanni* are *E. coli* strains ATCC8739 and *K. pneumoniae* ATCC11296 strains. Notice that these extracts from three animal *C. guanhumi, G. campestris,* and *T. hermanni* did not show any MBC on all bacteria. Ciprofloxacin used as a positive control, exhibited an antibacterial potential against all tested strains

and bactericidal effects on 75% of bacteria. Its activity is comparable to that of dried *R. jimi* extract.

Effects of the combinations of antibiotics with extracts

Tested extracts were associated with some commonly used antibiotics with the aim of making studied bacteria more susceptible. Calculation of the improvement activity factors (IAFs) gives an idea of the type of effects of these combinations which can be synergism (IAF≥2), indifference (IAF=1), or antagonism (IAF≤0.5). Results are shown in Tables 5-10. It was noted that synergistic effects were observed in the majority of cases between extracts and antibiotics with IAFs values ranging from 2 to 256. In the presence of dried *G. campestris* the activities of 56% of antibiotics (Ciprofloxacin, Doxycycline, Flucloxacillin, Ofloxacin, and Azithromycin) increased against at least 70% of the studied bacteria, and the activities of other antibiotics improved on less than 50% of the bacteria. Ciprofloxacin activity was improved more than that of the other antibiotics, as IAFs≥16 were obtained in the majority of cases (Table 5). Bacterial strains like *E. coli* ATCC8739, *E. aerogenes* (ATCC13048, and CM64), *K. pneumoniae* ATCC11296, and *P. stuartii* ATCC29916 were more susceptible to the combination of this extract and many antibiotics. Fresh extract from *T. hermanni* enhanced the activity of 33% of antibiotics including Ciprofloxacin, Doxycycline, and Ofloxacin against more than 70% of bacteria (Table 6). This extract most potentiated the activities of the other antibiotics than extract from dried *G. campestris*. In presence of this *T. hermanni* extract, the susceptibility of four bacteria including *E. coli* (ATCC8739 and AG102), *K. pneumoniae* ATCC11296 and *P. aeruginosa* PA01 was most pronounced against the majority of antibiotics. Moreover, no antagonistic effect was observed between these two extracts and all antibiotics. Tables 7 and 8 showed that dried and fresh extracts of *C. guanhumi* at all MICs enhanced the activities of 67% and 56% of antibiotics, respectively against almost 70% of bacteria. They also most potentiated the activity of Ciprofloxacin as the MICs values of their combinations highly decreased than those of this antibiotic alone against the majority of bacteria. Four bacterial strains such as *E. coli* (ATCC8739 and AG102), *K. pneumoniae* ATCC11296 and *P. aeruginosa* PA01 were most susceptible to the combinations with dried extract (Table 7) while in the presence of fresh extract (Table 8), the pathogenic power of three bacterial strains including *E. coli* AG102, *K. pneumoniae* ATCC11296 and *P. aeruginosa* PA01 highly decreased. However, an antagonistic effect was obtained between the dried extract and Oxacillin against *K. pneumoniae* Kp55 on the one hand and between the fresh extract of this animal and Flucloxacillin against *P. stuartii* NEA16 on the other hand. At all concentrations (MIC/2 and MIC/4), dried and fresh extracts of *R. jimi* potentiated the antibacterial activities of 89% and 78% of antibiotics, respectively against almost 70% of studied strains with IAF values ranging from 2 to 256 for dried extracts (Table 9) and 2 to 128 for fresh extracts (Table 10). Each of these two extracts highly improved the activities of Oxacillin, Gentamicin, Erythromycin and Ciprofloxacin against the majority of bacteria. Except for *E. aerogenes* ATCC13048 and *P. aeruginosa* PA124, all studied bacteria were more susceptible vis-à-vis the combinations between dried extract and the majority of antibiotics, meanwhile, combinations with fresh extract highly reduced the virulence of 50% of bacteria that are *E. aerogenes* CM64, *K. pneumoniae* (ATCC11296 and Kp55) and *P. stuartii* (ATCC29916 and NEA16). Moreover, no antagonistic effect was not obtained with these dried and fresh extracts of *R. jimi* and all the used antibiotics. Therefore, many cases of indifference effects were selectively observed between all tested extracts and antibiotics.

Discussion

Bacterial infections caused by MDR Gram-negative pathogens remain a global public health concern as therapeutic options for their treatment are dwindling. The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This is true for agents used in the treatment of bacterial, fungal, parasitic, and viral infections and for the treatment of chronic diseases such as cancer and diabetes; it applies to ailments caused or suffered by any living organism, including humans, animals, fish, plants, insects, etc. A wide range of biochemical and physiological mechanisms may be responsible for resistance [43]. The problem of microbial resistance to conventional antibiotics can therefore be solved by using natural substances of plant or animal origin that are a potential source of antimicrobials and are responsible for the antimicrobial activities of plants or animals. All animals contain proteins, although not all animal peptides are antimicrobial. Indeed, the results obtained in this work show that *Rhinella jimi* extracts contain, in addition to peptides or proteins, secondary metabolites such as alkaloids, flavonoids, tannins, and steroids. This would explain its antibacterial potential compared to other animal extracts used. Similarly, the presence of alkaloids in the *Cardisoma guanhumi* extract makes it more active than the *Gryllus campestris* and *Testudo hermanni* extracts, which are devoid of secondary metabolites. Indeed, work on *R. jimi* and several other animal species of the same family has revealed significant antibacterial activities against some susceptible and resistant strains of *Echerichia coli* and *Staphyloccocus aureus* [44,45]. Antimicrobial peptides are one of the key elements of the innate immune system, serving to defend multicellular organisms [46,47]. The very structure of peptides gives them some essential characteristics for antimicrobial action such as their small size (12 to 15 amino acids) and their amphiphilic, anionic, and cationic characteristics [47-49]. More than 750 antimicrobial cationic peptides (ACPs) from various animal organisms, including vertebrates and invertebrates, have been isolated to date [50,51]. In addition, most ACPs have a broad spectrum of activity since they cover the majority of bacterial and fungal species, including those pathogenic to humans, enveloped viruses, and protozoa [52]. In multicellular organisms, ACPs are effective molecules of innate immunity. Their action in antimicrobial control can be exerted in two distinct ways, either by an antibiotic type of microorganism with a view to destroying them by a specific mechanism, or by an immunomodulatory activity involving the recruitment and activation of immune cells at the site of the infection [52]. Furthermore, the spectrum of activity of anionic antimicrobial peptides is broad as they act against both Gram-positive and Gram-negative bacteria. The interactions established between the anionic peptides and the bacterial plasma membrane seem to be essential for this activity. To facilitate these interactions, they adopt an amphiphilic structure and require the intervention of cofactors, which are usually metal ions such as zinc and copper [53, 54]. The binding of peptides to ions leads to the formation of salts, which can interact with the anionic constituents of microbial membranes, such as teichoic acids in Gram-positive or lipopolysaccharides in Gram-negative bacteria. The mechanism by which these peptides exert their activity and cause cell death, however, is not well understood [53, 54]. The accessibility of antimicrobial peptides to several targets is a significant advantage in combining the development of bacterial resistance. Indeed, the specificity of action towards a single target that is present in some molecules such as conventional antibiotics, favours the

establishment and selection of resistant strains. Moderate activity on several targets, therefore, seems to be a favourable condition for limiting resistance [55]. It has been shown that Invertebrates show elevated immunity after an infection has been cleared [56]. Their immune systems consist of constitutive and inducible defense mechanisms [57-59]. Important components are antimicrobial peptides and proteins, such as lysozyme, which are inducible above constitutive levels for many days after bacterial infection [60-62].

Constituents from natural substances are increasingly combined with commonly used antibiotics to improve the antimicrobial or therapeutic effectiveness of these antibiotics. Combination therapy has been proposed as a novel strategy to maximize antimicrobial efficacy against MDR pathogens and suppress the spread of resistance. Numerous extracts and compounds isolated from medicinal and food plants have shown synergistic effects with conventional antibiotics by significantly reducing their minimal inhibitory concentrations against several bacterial strains. Similarly, the potentiating effect of antibiotics by methanol extracts from some vertebrate and invertebrate animals has been demonstrated [66-67]. The results of the present study show that all the tested samples selectively potentiated the activity of all the antibiotics used, but with varying degrees of efficiency. This can be explained by the fact that some of these extracts, in this case, those of *R. jimi*, contain active principles that act as efflux pump inhibitors, thus increasing the intracellular concentrations of antibiotics, given that the bacteria used are multidrug-resistant strains with a tripartite efflux pump system known as resistance-nodulation cell division (RND), of which the Mex and Acr systems for example, in *P. aeruginosa* and *E. coli* respectively [68-70]. The increase in the activity of antibiotics in the presence of tested animal extracts would also result from the synergistic effects between these two substances which would act at different sites at the level of the bacteria cell by mechanisms of which the most important are, the weakening of the wall or cytoplasmic membrane, the inhibition of the synthesis of proteins, of genetic material, of ribosomes or of folic acid metabolism [71,72]. This property could be attributed to antimicrobial peptides, or any other secondary metabolites contained in the meat extracts used. However, the antimicrobial activity of the studied animal extracts has not yet been evaluated. To the best of our knowledge, this work, therefore, constitutes the first investigation into their antimicrobial potential, particularly against MDR bacterial strains.

Table 1. Information on the studied animal samples and their extraction yields

Animal samples	Family	Extraction yields (%)	Traditional uses	Biological activities	Identified or isolated bioactive compounds
Gryllus campestris Linnaeus	Grillidae	12.05	Used to treat itching and cutaneous inflammations; it also refreshes the memory [73-75]	Antimicrobial and immune system inducer [60,62]	Peptides and proteins, such as lysozyme [60,62]
Testudo hermanni, Mojsisovics	Testudinidae	2.36	Used in the treatment of cutaneous infections, rheumatism, and arthritis [73,76]	Antimicrobial activities of aqueous extracts used in indigenous medicine [77]	Not reported but peptides or proteins were identified in the present study
Cardisoma guanhumi Latreille	Gecarcinidae	2.35	Elderly in Malaysia tend to consume mud crabs' soup as traditional remedy and folk medicine for the purpose of reducing the symptoms of dengue fever. It is used to treat toxo-dietary infections and constipation [74,75,78]	Peptides in mud crabs from the genus of *Scyllia* have antimicrobial activity against some Gram-positive and Gram-negative bacteria, and showed antioxidant activity [79]	Peptides [79]
Rhinella jimi Stevaux	Arthroleptidae	15.6	It is used in neoplastic treatment, cutaneous itching and infections [73, 80, 81]	Has antibacterial activity against *Echerichia coli* and *Staphyloccocus aureus* and anticancer activitiy [82,83]	Not reported but peptides and alkaloids were identified in this study

NT: not reported

Table 2. Features of the studied bacteria

Species	Types	Characteristics	References
Escherichia coli	ATCC8739	Reference strain	[84]
	AG100A	*E. coli* K-12 expressing △acrAB: KAN^r	[85]
	AG102	△acrAB mutant AG100, owing acrF gene markedly over expressed; TET^r	[86]
	AG100A$_{Tet}$	△acrAB mutant AG100, with over-expressing *acrF* gene; TET^r	[87]
	W3110	Wild type *E. coli* K-12	
	MC4100	Wild type *E. coli* expressed ABC pumps KAN^r	[88]
Enterobacter aerogenes	ATCC13048	Reference strain	[84]
	EA27	Clinical MDR isolate exhibiting energy-dependent norfloxacin and chloramphenicol efflux with KAN^r, AMP^r, NAL^r, STR^r, TET^r	[89,90]
	EA289	KAN sensitive derivative of EA27	
	EA294	EA289 expressing acrA: KAN^r	[90]
	EA298	EA289 expressing *tolC* : KAN^r	[90,91]
Klebsiella pneumoniae	ATCC11296	Reference strain	
	Kp55	Clinical MDR isolate, TET^r, AMP^r, ATM^r, CEF^r	[84]
	Kp63	Clinical MDR isolate, TET^r, CHL^r, AMP^r, ATM^r	[82]
Providencia stuartii	NEA16	Clinical MDR isolate, AcrAB-TolC	[84]
	PS299645	Clinical MDR isolate, AcrAB-TolC associated to types OMPF and OMPC porines	
Pseudomonas aeruginosa	PA 01	Reference strain	[84]
	PA 124	Clinical MDR isolate, expressing *MexAB-OprM*	[85]

KANr, TETr, AMPr, NALr, STRr, ATMr, CEFr, CHLr : resistant (r) to kanamycin, tetracycline, ampicillin, nalidixic acid, streptomycin, aztreonam, cefepime, chloramphenicol, respectively; MDR : Multidrug-resistant ;. AcrAB-TolC, AcrAB and TolC are efflux pumps

Table 3. Zoochemical composition of animal extracts

Zoochemicals	Animal extracts					
	Gryllus campestris	*Testudo hermanni*	*Cardisoma guanhumi*		*Rhinella jimi*	
	Dried	Fresh	Dried	Fresh	Dried	Fresh
Alkaloids	-	-	+	+	+	+
Polyphenols	-	-	-	-	-	-
Flavonoids	-	-	-	-	+	+
Tannins	-	-	-	-	+	+
Steroids	-	-	-	-	+	+
Anthocyanins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Amino acids/ Peptides /Proteins	+	+	+	+	+	+

[-]: absence of zoochemicals [+]: presence of zoochemicals

Table 4. Minimal inhibitory and bactericidal concentrations of tested animal samples

Bacterial strains	**Animal extracts (µg/mL)**										**Ciprofloxacin (µg/mL)**		
	G. campestris	***T. hermanni***	***C. guanhumi***		***R. jimi***								
	Dried	**Fresh**	**Dried**	**Fresh**	**Dried**			**Fresh**					
	MIC	MIC	MIC	MIC	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Escherichia coli													
ATCC8739	512	1024	2048	1024	128	256	2	512	2048	4	1	4	4
AG100A	-	-	-	-	-	nt	nd	-	nt	nd	2	8	4
AG102	2048	-	-	-	2048	-	>1	-	nt	nd	4	8	2
AG100ATet	-	-	-	1024	128	512	4	1024	-	>2	16	32	2
MC4100	-	-	2048	2048	512	2048	4	1024	-	>2	2	4	2
W3110	2048	-	-	-	2048	-	>1	2048	-	>1	4	16	4
Enterobacter aerogenes													
ATCC13048	-	-	-	-	1024	-	>2	1024	-	>2	1	8	8
EA27	-	-	2048	-	512	1024	2	2048	-	>1	1	16	16
EA289	-	-	-	-	-	nt	nd	-	nt	nd	2	16	8
EA294	-	-	-	-	2048	-	>1	-	nt	nd	2	8	4
EA298	-	-	-	-	-	nt	nd	-	nt	nd	16	64	4
CM64	-	-	-	2048	1024	-	>2	512	2048	4	32	128	4
Klebsiella pneumoniae													
ATCC11296	-	1024	1024	-	512	1024	2	2048	-	>1	2	4	2
KP55	-	-	2048	-	2048	-	>1	2048	-	>1	32	128	4
KP63	-	-	1024	-	2048	-	>1	-	nt	nd	16	16	1
Providencia stuartii													
ATCC29916	-	-	-	-	1024	-	>2	-	nt	nd	1	8	8
PS2636	-	-	2048	-	2048	-	>1	-	nt	nd	2	8	4
NEA16	-	-	-	-	2048	-	>1	2048	-	>1	1	4	4
Pseudomonas aeruginosa													
PA01	-	-	2048	-	256	1024	4	2048	-	>1	8	32	4
PA124	-	-	-	-	512	1024	2	-	nt	nd	32	256	8
PSBS (%)	**15**	**10**	**40**	**20**	**85**			**55**			**100**		

(-): MIC or MBC>2048 µg/mL (extracts were not active or have very weak activity); MIC : minimal inhibitory concentration; MBC : minimal bactericidal concentration; R : MBC / MIC ratio (a sample is considered as bacteriostatic or bactericidal when this ratio is >4 or ≤4 respectively); PSBS : percentage of susceptible bacteria to substances; nt : not tested; nd : not determined (as no MIC and MBC values were not observed till 2048 µg/mL); Gc: *Gryllus campestris*; Th: *Testudo hermanni*; Cg: *Cardisoma guanhumi* ; Rj: *Rhinella jimi*; the activity of dried R. jimi is compared to that of positive control (ciprofloxacin) on some bacterial strains

Table 5. MICs of antibiotics in presence of dried *Gryllus campestris* extract at sub-inhibitory concentrations

Antibiotics	**MICs of extract**	**Bacterial strains and concentrations of antibiotics**										**PBS (%)**
		E. coli		***E. aerogenes***		***K. pneumoniae***		***P. aeruginosa***		***P. stuartii***		
		ATCC8739	**AG102**	**ATCC13048**	**CM64**	**ATCC11296**	**KP55**	**PA01**	**PA124**	**ATCC29916**	**NEA16**	
Oxacillin	0	32	32	64	64	64	2	64	32	64	64	
	MIC/2	8(4)	8(4)	64(1)	64(1)	64(1)	1(2)	64(1)	8(4)	32(2)	64(1)	50
	MIC/4	32(1)	32(1)	64(1)	64(1)	64(1)	1(2)	64(1)	16(2)	64(1)	64(1)	20
Thiamphenicol	0	2	2	4	4	32	16	2	4	16	8	
	MIC/2	0.5(4)	1(2)	4(1)	1(4)	32(1)	16(1)	2(1)	1(4)	2(8)	8(1)	50
	MIC/4	2(1)	1(2)	4(1)	2(2)	32(1)	16(1)	2(1)	1(4)	4(4)	8(1)	40
Gentamicin	0	2	1	16	8	1	2	16	4	16	8	
	MIC/2	1(2)	1(1)	0.25(64)	0.125(64)	0.5(2)	2(1)	16(1)	4(1)	0.25(64)	8(1)	50
	MIC/4	2(1)	1(1)	0.5(32)	0.25(32)	0.5(2)	2(1)	16(1)	4(1)	0.25(64)	8(1)	40
Erythromycin	0	1	2	16	16	1	8	16	2	16	16	
	MIC/2	0.125(8)	2(1)	16(1)	16(1)	1(1)	8(1)	16(1)	2(1)	0.5(32)	1(16)	30
	MIC/4	1(1)	2(1)	16(1)	16(1)	1(1)	8(1)	16(1)	2(1)	0.5(32)	2(8)	20
Ciprofloxacin	0	1	4	1	32	2	32	8	32	4	16	
	MIC/2	0.125(8)	2(2)	0.125(8)	0.5(8)	0.125(16)	2(1)	0.125(64)	8(4)	0.125(32)	0.25(16)	**90**
	MIC/4	0.25(4)	2(2)	0.25(4)	1(4)	0.125(16)	2(1)	0.25(32)	16(2)	0.5(8)	0.25(16)	**90**
Doxycycline	0	2	8	2	2	16	8	4	16	2	16	
	MIC/2	0.125(8)	0.5(16)	0.125(16)	0.25(8)	0.5(32)	8(1)	2(2)	4(4)	0.5(4)	16(1)	**80**
	MIC/4	0.25(4)	1(8)	0.125(16)	0.5(4)	0.5(32)	8(1)	2(2)	8(2)	1(2)	16(1)	**80**
Flucloxacillin	0	16	2	32	4	32	8	4	32	2	32	
	MIC/2	0.25(64)	0.25(8)	16(2)	1(4)	1(32)	8(1)	0.5(8)	32(1)	1(2)	32(1)	**70**
	MIC/4	0.5(32)	0.25(8)	32(1)	2(2)	1(32)	8(1)	1(4)	32(1)	1(2)	32(1)	60
Ofloxacin	0	2	2	32	16	8	2	4	2	2	16	
	MIC/2	0.25(8)	2(1)	0.5(64)	0.25(64)	0.25(32)	0.5(4)	0.5(8)	0.25(8)	1(2)	16(1)	**80**
	MIC/4	0.25(8)	2(1)	1(32)	0.25(64)	0.5(16)	1(2)	0.5(8)	0.5(4)	1(2)	16(1)	**80**
Azithromycin	0	4	16	16	16	4	4	4	4	4	16	
	MIC/2	0.5(8)	16(1)	1(16)	0.25(64)	0.5(8)	1(4)	0.5(8)	4(1)	1(4)	16(1)	**70**
	MIC/4	1(4)	16(1)	1(16)	0.5(32)	1(4)	4(1)	1(4)	4(1)	1(4)	16(1)	60

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of Ciprofloxacin, Doxycycline and Ofloxacin were more potentiated at all concentrations of extract

Table 6. MICs of antibiotics in presence of fresh *Testudo hermanni* extract at sub-inhibitory concentrations

Antibiotics	MICs of extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	PA01	PA124	ATCC29916	NEA166	
Oxacillin	0	32	32	64	64	64	2	64	32	64	64	
	MIC/2	0.5(64)	8(4)	1(64)	64(1)	1(64)	2(1)	1(64)	32(1)	64(1)	1(64)	60
	MIC/4	0.5(64)	32(1)	1(64)	64(1)	1(64)	2(1)	1(64)	32(1)	64(1)	1(64)	50
Thiamphenicol	0	2	2	4	4	32	16	2	4	16	8	
	MIC/2	1(2)	0.25(8)	4(1)	4(1)	32(1)	4(4)	0.25(8)	4(1)	0.5(32)	8(1)	50
	MIC/4	2(1)	1(2)	4(1)	4(1)	32(1)	16(1)	1(2)	4(1)	1(16)	8(1)	30
Gentamicin	0	2	1	16	8	1	2	16	4	16	8	
	MIC/2	0.125(16)	0.125(8)	16(1)	1(8)	0.25(4)	2(1)	1(16)	0.5(8)	16(1)	8(1)	60
	MIC/4	0.125(16)	0.25(4)	16(1)	1(8)	0.5(2)	2(1)	1(16)	1(4)	16(1)	8(1)	60
Erythromycin	0	1	2	16	16	1	8	16	2	16	16	
	MIC/2	1(1)	0.125(16)	1(16)	16(1)	1(1)	0.5(16)	16(1)	2(1)	1(16)	1(16)	50
	MIC/4	1(1)	0.25(8)	2(8)	16(1)	1(1)	1(8)	16(1)	2(1)	1(16)	1(16)	50
Ciprofloxacin	0	1	4	1	32	2	32	8	32	4	16	
	MIC/2	0.125(8)	0.125(32)	0.125(4)	1(32)	0.125(16)	0.5(64)	0.125(64)	4(8)	0.25(16)	0.25(64)	**100**
	MIC/4	0.25(4)	0.25(16)	0.25(2)	0.5(8)	0.25(8)	1(32)	0.25(32)	4(8)	0.5(8)	0.5(32)	**100**
Doxycycline	0	2	8	2	2	16	8	4	16	2	16	
	MIC/2	0.125(16)	0.5(16)	0.25(8)	0.5(4)	0.5(32)	0.125(64)	0.25(16)	2(8)	1(2)	16(1)	**90**
	MIC/4	0.25(8)	1(8)	0.25(8)	0.5(4)	0.5(32)	0.25(32)	0.5(32)	4(4)	2(1)	16(1)	**80**
Flucloxacillin	0	16	2	32	4	32	8	4	32	2	32	
	MIC/2	1(16)	0.125(16)	32(1)	1(4)	0.5(64)	8(1)	0.5(32)	64(0.5)	1(2)	32(1)	60
	MIC/4	1(16)	0.25(8)	32(1)	1(4)	1(32)	8(1)	0.5(32)	64(0.5)	1(2)	32(1)	60
Ofloxacin	0	2	2	32	16	8	2	4	2	2	16	
	MIC/2	0.25(8)	0.25(8)	16(2)	0.25(64)	0.5(16)	0.5(4)	0.5(32)	1(2)	1(2)	0.5(32)	**100**
	MIC/4	0.5(4)	0.5(4)	16(2)	0.25(64)	1(8)	0.5(4)	0.5(32)	2(1)	1(2)	1(16)	**90**
Azithromycin	0	4	16	16	16	4	4	4	4	4	16	
	MIC/2	0.5(8)	0.5(32)	16(1)	0.25(64)	0.125(32)	4(1)	0.5(32)	2(2)	4(1)	16(1)	60
	MIC/4	0.5(8)	1(16)	16(1)	0.25(64)	0.125(32)	4(1)	0.5(8)	2(2)	4(1)	16(1)	60

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of Ciprofloxacin, Doxycycline and Ofloxacin were more potentiated at all concentrations of extract

Table 7. MICs of antibiotics in presence of dried *Cardisoma guanhumi* extract at sub-inhibitory concentrations

Antibiotics	MICs of extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	PA01	PA124	ATCC29916	NEA16	
Oxacillin	0	32	32	64	64	64	2	64	32	64	64	
	MIC/2	8(4)	8(4)	2(32)	64(1)	1(64)	4(0.5)	2(32)	32(1)	64(1)	1(64)	60
	MIC/4	61(2)	32(1)	2(32)	64(1)	4(16)	4(0.5)	4(16)	32(1)	64(1)	1(64)	50
Thiamphenicol	0	2	2	4	4	32	16	2	4	16	8	
	MIC/2	1(2)	0.5(8)	0.5(8)	4(1)	32(1)	4(4)	0.25(8)	4(1)	16(1)	1(8)	60
	MIC/4	2(1)	1(2)	1(4)	4(1)	32(1)	16(1)	1(2)	4(1)	16(1)	1(8)	40
Gentamicin	0	2	1	16	8	1	2	16	4	16	8	
	MIC/2	0.125(16)	0.5(2)	16(1)	1(8)	0.25(4)	2(1)	1(16)	2(4)	1(16)	1(8)	**70**
	MIC/4	0.125(16)	0.5(2)	16(1)	1(8)	0.5(2)	2(1)	1(16)	2(2)	1(16)	1(8)	**70**
Erythromycin	0	1	2	16	16	1	8	16	2	16	16	
	MIC/2	0.5(2)	0.125(16)	1(16)	16(1)	1(1)	0.5(16)	1(16)	0.5(4)	1(16)	1(16)	**80**
	MIC/4	0.5(2)	0.25(8)	2(8)	16(1)	1(1)	1(8)	1(16)	0.5(4)	2(8)	2(8)	**80**
Ciprofloxacin	0	1	4	1	32	2	32	8	32	4	16	
	MIC/2	<0.125(>8)	0.125(32)	0.25(2)	0.5(8)	0.125(64)	1(32)	0.125(64)	0.5(64)	0.25(64)	1(16)	**100**
	MIC/4	0.125(8)	0.25(16)	0.5(1)	0.5(8)	0.125(64)	2(16)	0.125(64)	0.5(64)	0.25(64)	1(16)	**90**
Doxycycline	0	2	8	2	2	16	8	4	16	2	16	
	MIC/2	1(2)	1(8)	0.25(8)	0.25(8)	0.5(32)	0.125(64)	0.25(16)	4(4)	1(2)	16(1)	**90**
	MIC/4	1(2)	1(8)	0.5(4)	0.5(4)	0.5(32)	0.5(16)	0.5(32)	8(2)	1(2)	16(1)	**90**
Flucloxacillin	0	16	2	32	4	32	8	4	32	2	32	
	MIC/2	1(16)	0.5(4)	4(8)	1(4)	0.5(64)	8(1)	0.5(32)	8(4)	2(1)	32(1)	**70**
	MIC/4	2(8)	1(2)	8(4)	1(4)	1(32)	8(1)	0.5(32)	16(2)	2(1)	32(1)	**70**
Ofloxacin	0	2	2	32	16	8	2	4	2	2	16	
	MIC/2	0.125(16)	0.5(4)	1(32)	0.25(64)	0.5(16)	0.5(4)	0.5(32)	2(1)	1(2)	16(1)	**80**
	MIC/4	0.25(8)	0.5(4)	1(32)	0.5(32)	1(8)	0.5(4)	0.5(32)	2(1)	1(2)	16(1)	**80**
Azithromycin	0	4	16	16	16	4	4	4	4	4	16	
	MIC/2	0.25(16)	16(1)	16(1)	0.25(64)	0.25(16)	4(1)	0.25(16)	4(1)	4(1)	16(1)	40
	MIC/4	0.5(8)	16(1)	16(1)	1(16)	0.25(16)	4(1)	1(4)	4(1)	4(1)	16(1)	40

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of Gentamicin, Erythromycin, Ciprofloxacin, Doxycycline and Ofloxacin were more potentiated at all concentrations of extract

Table 8. MICs of antibiotics in presence of fresh *Cardisoma guanhumi* extract at sub-inhibitory concentrations

Antibiotics	MICs of extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	PA01	PA124	ATCC29916	NEA16	
Oxacillin	0	32	32	64	64	64	2	64	32	64	64	
	MIC/2	32(1)	8(4)	64(1)	32(2)	1(64)	1(2)	64(1)	32(1)	64(1)	64(1)	40
	MIC/4	32(1)	32(1)	64(1)	64(1)	1(64)	1(2)	64(1)	32(1)	64(1)	64(1)	30
Thiamphenicol	0	2	2	4	4	32	16	2	4	16	8	
	MIC/2	0.5(4)	1(2)	2(2)	2(2)	32(1)	16(1)	2(1)	4(1)	2(8)	8(1)	50
	MIC/4	1(2)	2(1)	4(1)	2(2)	32(1)	16(1)	2(1)	4(1)	4(4)	8(1)	30
Gentamicin	0	2	1	16	8	1	2	16	4	16	8	
	MIC/2	2(1)	0.125(8)	16(1)	8(1)	0.5(2)	2(1)	0.25(64)	0.5(8)	16(1)	8(1)	40
	MIC/4	2(1)	0.125(8)	16(1)	8(1)	1(1)	2(1)	0.5(32)	1(4)	16(1)	8(1)	30
Erythromycin	0	1	2	16	16	1	8	16	2	16	16	
	MIC/2	0.25(4)	0.125(16)	4(4)	2(8)	1(1)	8(1)	0.5(32)	1(2)	0.5(32)	1(16)	**80**
	MIC/4	0.5(2)	0.25(8)	8(2)	2(8)	1(1)	8(1)	0.5(32)	1(2)	0.5(32)	2(8)	**80**
Ciprofloxacin	0	1	4	1	32	2	32	8	32	4	16	
	MIC/2	0.125(8)	0.125(32)	0.125(8)	1(32)	0.125(16)	2(1)	0.125(64)	0.5(64)	0.25(64)	0.25(64)	**90**
	MIC/4	0.25(4)	0.125(32)	0.25(4)	2(16)	0.25(8)	2(1)	0.25(32)	1(32)	0.25(64)	0.25(64)	**90**
Doxycycline	0	2	8	2	2	16	8	4	16	2	16	
	MIC/2	0.25(8)	0.25(32)	0.5(4)	0.5(4)	0.5(32)	8(1)	1(4)	4(4)	2(1)	16(1)	**70**
	MIC/4	1(2)	0.25(32)	0.5(4)	1(2)	0.5(32)	8(1)	2(2)	4(4)	2(1)	16(1)	**70**
Flucloxacillin	0	16	2	32	4	32	8	4	32	2	32	
	MIC/2	16(1)	0.25(8)	32(1)	0.5(8)	1(32)	8(1)	1(4)	32(1)	1(2)	64(0.5)	50
	MIC/4	16(1)	0.25(8)	32(1)	1(4)	1(32)	8(1)	1(4)	32(1)	1(2)	64(0.5)	50
Ofloxacin	0	2	2	32	16	8	2	4	2	2	16	
	MIC/2	0.25(8)	0.25(8)	0.5(64)	0.25(64)	0.5(16)	1(2)	0.25(16)	1(2)	1(2)	16(1)	**90**
	MIC/4	0.5(4)	0.25(8)	0.5(64)	0.5(32)	0.5(16)	1(2)	0.25(16)	1(2)	1(2)	16(1)	**90**
Azithromycin	0	4	16	16	16	4	4	4	4	4	16	
	MIC/2	0.25(16)	0.5(32)	1(16)	0.25(64)	0.5(8)	1(4)	0.5(8)	1(4)	1(4)	16(1)	**90**
	MIC/4	0.5(8)	1(16)	2(8)	0.5(32)	0.5(8)	4(1)	1(4)	2(2)	1(4)	16(1)	**90**

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of Erythromycin, Ciprofloxacin, Doxycycline and Ofloxacin were more potentiated at all concentrations of extract

Table 9. MICs of antibiotics in presence of dried *Rhinella jimi* extract at sub-inhibitory concentrations

Antibiotics	MICs of extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	PA01	PA124	ATCC29916	NEA16	
Oxacillin	0	32	32	64	64	64	2	64	32	64	64	
	MIC/2	0.25(128)	0.125(256)	16(4)	1(64)	16(4)	0.125(16)	0.5(128)	1(32)	0.25(256)	2(32)	**100**
	MIC/4	0.5(64)	0.25(128)	32(2)	2(32)	16(4)	0.25(8)	2(32)	2(16)	2(32)	8(8)	**100**
Thiamphenicol	0	2	2	4	4	32	16	2	4	16	8	
	MIC/2	1(2)	0.125(16)	4(1)	4(1)	8(4)	2(8)	0.25(8)	0.5(8)	16(1)	8(1)	60
	MIC/4	1(2)	0.5(4)	4(1)	4(1)	8(4)	2(8)	1(2)	1(4)	16(1)	8(1)	60
Gentamicin	0	2	1	16	8	1	2	16	4	16	8	
	MIC/2	0.125(16)	1(2)	0.25(64)	0.125(64)	0.125(8)	0.25(8)	0.25(64)	0.125(32)	0.5(32)	8(1)	**90**
	MIC/4	0.25(8)	2(1)	1(16)	0.25(32)	0.125(8)	0.5(4)	0.5(32)	0.25(16)	1(16)	8(1)	**80**
Erythromycin	0	1	2	16	16	1	8	16	2	16	16	
	MIC/2	0.125(8)	0.25(8)	0.25(64)	0.25(64)	0.125(8)	0.25(32)	0.5(32)	0.125(16)	0.125(128)	1(16)	**100**
	MIC/4	0.25(4)	0.5(4)	0.25(64)	0.5(32)	0.5(2)	0.5(16)	1(16)	0.25(8)	0.25(64)	1(16)	**100**
Ciprofloxacin	0	1	4	1	32	2	32	8	32	4	16	
	MIC/2	0.125(8)	0.25(16)	0.25(4)	2(16)	0.125(16)	1(32)	0.25(64)	1(32)	0.125(32)	1(16)	**100**
	MIC/4	0.25(4)	0.25(16)	0.25(4)	2(16)	0.25(8)	2(16)	1(8)	2(16)	0.25(16)	2(8)	**100**
Doxycycline	0	2	8	2	2	16	8	4	16	2	16	
	MIC/2	0.125(16)	0.5(16)	2(1)	0.25(8)	2(8)	0.5(16)	4(1)	4(4)	0.125(16)	0.5(32)	**80**
	MIC/4	0.125(16)	2(4)	2(1)	0.25(8)	4(4)	2(4)	4(1)	4(4)	0.25(8)	1(16)	**80**
Flucloxacillin	0	16	2	32	4	32	8	4	32	2	32	
	MIC/2	2(8)	0.25(8)	0.5(64)	0.125(32)	4(8)	0.5(16)	1(4)	32(1)	1(2)	2(16)	**90**
	MIC/4	4(4)	0.25(8)	2(16)	0.25(16)	8(4)	8(4)	2(2)	32(1)	1(2)	2(16)	**90**
Ofloxacin	0	2	2	32	16	8	2	4	2	2	16	
	MIC/2	0.5(4)	0.125(16)	2(16)	0.25(64)	0.5(16)	2(1)	0.125(16)	1(2)	0.125(16)	16(1)	**90**
	MIC/4	0.5(4)	0.25(8)	8(4)	0.5(32)	1(8)	2(1)	0.25(8)	1(2)	0.25(8)	16(1)	**80**
Azithromycin	0	4	16	16	16	4	4	4	4	4	16	
	MIC/2	0.5(8)	16(1)	4(4)	1(16)	0.5(8)	0.25(16)	0.25(16)	4(1)	4(1)	1(16)	**70**
	MIC/4	1(4)	16(1)	4(4)	2(8)	0.5(8)	2(2)	0.5(8)	4(1)	4(1)	2(8)	**70**

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of almost all

Table 10. MICs of antibiotics in presence of fresh *Rhinella jimi* extract at sub-inhibitory concentrations

Antibiotics	MICs of extract	Bacterial strains and concentrations of antibiotics										PBS (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*P. stuartii*		
		ATCC8739	AG102	ATCC13048	CM64	ATCC11296	KP55	PA01	PA124	ATCC29916	NEA16	
	0	32	32	64	64	64	2	64	32	64	64	
Oxacillin	MIC/2	0.5(64)	0.25(128)	64(1)	2(32)	1(64)	0.125(16)	1(64)	8(4)	0.5(128)	1(64)	**90**
	MIC/4	2(16)	1(32)	64(1)	4(16)	4(16)	0.5(4)	2(32)	16(2)	2(32)	2(32)	**90**
	0	2	2	4	4	32	16	2	4	16	8	
Thiamphenicol	MIC/2	2(1)	1(2)	4(1)	4(1)	2(16)	8(2)	1(2)	2(2)	16(1)	0.5(16)	60
	MIC/4	2(1)	1(2)	4(1)	4(1)	2(16)	16(1)	1(2)	2(2)	16(1)	1(8)	50
	0	2	1	16	8	1	2	16	4	16	8	
Gentamicin	MIC/2	0.125(16)	0.25(4)	16(1)	8(1)	0.25(4)	0.125(16)	16(1)	0.125(32)	0.5(32)	0.25(32)	**70**
	MIC/4	0.125(16)	0.5(2)	16(1)	8(1)	0.5(2)	0.125(16)	16(1)	0.125(32)	1(16)	0.25(32)	**70**
	0	1	2	16	16	1	8	16	2	16	16	
Erythromycin	MIC/2	1(1)	2(1)	0.5(32)	0.25(64)	0.125(8)	0.125(64)	1(16)	1(2)	0.25(64)	0.5(32)	**90**
	MIC/4	1(1)	2(1)	1(16)	0.5(32)	0.125(8)	0.25(32)	2(8)	1(2)	0.5(32)	0.5(32)	**80**
	0	1	4	1	32	2	32	8	32	4	16	
Ciprofloxacin	MIC/2	0.125(8)	2(1)	0.125(8)	0.5(64)	0.125(16)	0.5(64)	0.125(64)	4(8)	0.25(16)	0.25(64)	**90**
	MIC/4	0.125(8)	2(1)	0.25(4)	1(32)	0.125(16)	0.5(64)	0.25(32)	8(4)	0.25(16)	0.25(64)	**90**
	0	2	8	2	2	16	8	4	16	2	16	
Doxycycline	MIC/2	0.25(8)	0.25(32)	2(1)	0.25(8)	0.5(32)	0.25(32)	1(4)	2(8)	2(1)	16(1)	**70**
	MIC/4	0.25(8)	0.25(32)	2(1)	0.5(4)	1(16)	0.5(16)	1(4)	2(8)	2(1)	16(1)	**70**
	0	16	2	32	4	32	8	4	32	2	32	
Flucloxacillin	MIC/2	8(2)	0.5(4)	32(1)	0.5(8)	4(8)	0.25(32)	1(4)	32(1)	2(1)	32(1)	**60**
	MIC/4	8(2)	0.5(4)	32(1)	0.5(8)	8(4)	0.5(16)	1(4)	32(1)	2(1)	32(1)	60
	0	2	2	32	16	8	2	4	2	2	16	
Ofloxacin	MIC/2	2(1)	0.25(8)	0.5(64)	0.25(64)	0.5(16)	0.5(4)	0.25(16)	0.25(8)	0.125(16)	1(16)	**90**
	MIC/4	2(1)	0.25(8)	1(32)	0.25(64)	0.5(16)	0.5(4)	0.5(8)	0.5(4)	0.25(8)	1(16)	**90**
	0	4	16	16	16	4	4	4	4	4	16	
Azithromycin	MIC/2	0.5(8)	1(16)	0.5(32)	0.25(64)	0.5(8)	0.5(8)	0.5(8)	0.5(8)	4(1)	8(2)	**90**
	MIC/4	1(4)	4(4)	0.5(32)	0.25(64)	1(4)	1(4)	0.5(8)	0.5(8)	4(1)	16(1)	**80**

PBS : percentage of bacterial susceptibility; The numbers in parenthesis represent the improvement activity factors (IAF) [there is synergism when IAF≥2, indifference when IAF=1 and antagonism when IAF≤0.5]; IAF values were obtained by calculating the MIC of antibiotic alone over MIC of the combination; 0: MICs values of antibiotics tested alone; The MIC of extract sample is those showed in Table 4; *E. coli: Escherichia coli; E. aerogenes: Enterobacter aerogenes; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; P. stuartii: Providencia stuartii*; the activities of almost all the antibiotics were more potentiated at all concentrations of extract

Conclusion

This study revealed that *R. jimi* is the most promising animal extract, as it was active against most of the studied bacteria. All tested animal extracts enhanced the activity of almost all antibiotics used against several bacteria but those from *R. jimi* had the most potential compared to the other extracts. It would therefore constitute an alternative in the control and treatment of infections caused by MDR bacteria. Finally, the present study provides basic information about the antimicrobial potential of the studied animals.

Abbreviations

ACPs, antimicrobial cationic peptides; ATCC, American type culture collection; DMSO, dimethylsulfoxide; EPIs, efflux pumps inhibitors; EY, extractive yield; INT, p-Iodonitrotetrazolium chloride; MBC, minimal bactericidal concentration; MDR, multidrug resistant; MIC, minimal inhibitory concentration; PBS, percentage of bacterial susceptibility; PSBS, percentage of susceptible bacteria to substances; RND, resistance-nodulation cell division; IAF, improvement activity factors

Authors' Contribution

MGGF collected and extracted animal samples and performed the antibacterial assays. CMNN performed the zoochemical screening. INB, BENW, and PN performed the minimal inhibitory concentrations of antibiotics in combination with extracts. SBT wrote the manuscript. VK and ATM designed the study and supervised the work. VK provided the bacterial strains and facilities for antibacterial assays. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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References

1. Dehkordi FS, Yazdani F, Mozafari J et al. 2014. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. *BMC Res Notes* 7, 217.
2. Dormanesh B, Safarpoor Dehkordi F, Hosseini S, et al. 2014. Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian paediatric patients. *Iran Red Crescent Med J.* 16, e14627
3. Alekshun MN, Levy SB. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell*., 128(6),1037–1050
4. Davin-Regli A, Bolla JM, James CE, Lavigne JP, Chevalier J, Garnotel E, Molitor A. 2008. Membrane permeability and regulation of druginflux and efflux" in enterobacterial pathogens. *Curr Drug Targets* 9(9), 750–759
5. Nikaido H. 2009. Multidrug resistance in bacteria. *Annu Rev Biochem* 78, 119–146
6. Odonkor T Stephen and Addo K Kennedy, 2011. Bacteria resistance to antibiotics: recent trends and challenge. *Int J of Biol Med Res.* 2(4), 1204-1210.
7. Momtaz H, Safarpoor Dehkordi F, Rahimi E et al. 2013. Virulence genes and antimicrobial resistance profiles of *Staphylococcus aureus* isolated from chicken meat in Iffahan province. *Iran J Appl Poult Res*. 22, 913-921
8. Chaleshtori Hosseini Seyede Asma, Kachoie Ataie Mehrdad, Jasi Hashemi Seyed Mojtaba, 2017. Antibacterial effects of the methanolic extract of Glycine max (soybean). *Microbiol Res.* 8, 7319
9. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ 2006. Global burden of disease and risk factors. Washington. DC, USA: IBRD/ The World Bank and Oxford University Press, http://files.dcp2.org/pdf/GBD/ GBDFM.pdf
10. Shebl RI, Mohamed AF, Ali AE, Amin MA. 2012. Antimicrobial profile of selected snake venoms and their associated enzymatic activities. *British Microbiol Res J.* 2(4), 251-263
11. Ngaffo CMN, Tankeo SB, Guefack MGF, Nayim P, Wamba BEN, Kuete V , Mbaveng AT. 2021. Phytochemical analysis and antibiotic-modulating activity of *Cocos nucifera, Glycine max* and *Musa sapientum* methanol extracts against multidrug resistant Gram-negative bacteria. *Investigational Medicinal Chemistry and Pharmacology* 4(2), 53.
12. Kuete V, Sandjo LP. 2012. Isobavachalcone: an overview. *Chin J Integr Med*, 18(7):543-547.
13. Mbaveng AT, Kuete V, Efferth T. 2017. Potential of Central, Eastern and Western Africa medicinal plants for cancer therapy: spotlight on resistant cells and molecular targets. *Front pharmacol.* 8, 343.
14. Kuete V, Sandjo LP, Djeussi DE, Zeino M, Kwamou GM, Ngadjui B, Efferth. T2014. Cytotoxic flavonoids and isoflavonoids from *Erythrina sigmoidea* towards multi-factorial drug resistant cancer cells. *Invest New Drugs.* 32,1053–1062.
15. Kuete V, Nkuete AHL, Mbaveng AT, Wiench B, Wabo HK, Tane P, Efferth T. 2014. Cytotoxicity and modes of action of 4′-hydroxy-2′,6′-dimethoxychalcone and other flavonoids toward drug-sensitive and multidrug-resistant cancer cell lines. *Phytomedicine* 21(12), 1651-1657.
16. Ngongang FCM, Fankam AG, Mbaveng AT, Wamba BEN, Nayim P, Beng VP and Kuete V. 2020. Methanol Extracts from *Manilkara zapota* with moderate antibacterial activity displayed strong antibiotic-modulating effects against multidrug-resistant phenotypes. Investigational Medicinal Chemistry and Pharmacology **3(1)**, 37.
17. Mbaveng AT, Ndontsa BL, Kuete V, Nguekeu YMM, Celik I, Mbouangouere R, Tane P, Efferth T. 2018. A naturally occuring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death. *Phytomedicine* 43, 78-85.
18. Dzoyem JP, McGaw LJ, Kuete V, Bakowsky U. 2017. Chapter 9 - Anti-inflammatory and Anti-nociceptive Activities of African Medicinal Spices and Vegetables. In: *Medicinal Spices and Vegetables from Africa.* edn.: Academic Press. Pp. 239-270.
19. Kuete V, Tangmouo JG, Penlap Beng V, Ngounou FN, Lontsi D. 2006. Antimicrobial activity of the methanolic extract from the stem bark of *Tridesmostemon omphalocarpoides* (Sapotaceae). *Jf Ethnopharmacol.*, 104(1–2), 5-11.
20. Lacmata ST, Kuete V, Dzoyem JP, Tankeo SB, Teke GN, Kuiate JR, Pages JM. 2012. Antibacterial activities of selected Cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. *Evid Based Complement Alternat Med* 2012, 623723.
21. Mbaveng AT, Sandjo LP, Tankeo SB, Ndifor AR, Pantaleon A, Nagdjui BT, Kuete V. 2015. Antibacterial activity of nineteen selected natural products against multi-drug resistant Gram-negative phenotypes. *Springerplus*, **4**, 823.
22. Kuete V, Sandjo LP, Mbaveng AT, Seukep JA, Ngadjui BT, Efferth T. 2012´5. Cytotoxicity of selected Cameroonian medicinal plants and *Nauclea pobeguinii* towards multi-factorial drug-resistant cancer cells. *BMC Complement Altern Med* 15, 309.
23. Nono EC, Mkounga P, Kuete V, Marat K, Hultin PG, Nkengfack AE. 2010. Pycnanthulignenes A-D, antimicrobial cyclolignene derivatives from the roots of *Pycnanthus angolensis*. *J Nat Prod*. 73(2), 213-216.
24. Fankam AG, Kuiate JR, Kuete V. 2015. Antibacterial and antibiotic resistance modifying activity of the extracts from *Allanblackia gabonensis, Combretum molle* and *Gladiolus quartinianus* against Gram-negative bacteria including multi-drug resistant phenotypes. *BMC Complement Altern Med.* 15.206.
25. Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocaru D, Dzoyem JP, Kuete V. 2013. *In vitro* antibacterial and antibiotic-potentiation activities of four edible plants against multidrug-resistant Gram-negative species. *BMC Complement Altern Med*. 13, 190.
26. Tekwu EM, Askun T, Kuete V, Nkengfack AE, Nyasse B, Etoa FX, Beng VP. 2012. Antibacterial activity of selected Cameroonian dietary spices ethno-medically used against strains of *Mycobacterium tuberculosis*. *J Ethnopharmacol* 142, 374-382.
27. Komguem J, Meli AL, Manfouo RN, Lontsi D, Ngounou FN, Kuete V, Kamdem HW, Tane P, Ngadjui BT, Sondengam BL, Tane P, Connolly, J. D. 2005. Xanthones from *Garcinia smeathmannii* (Oliver) and their antimicrobial activity. *Phytochemistry* 66, 1713-1717.
28. Tchinda, C.F., Voukeng, I.K., Veronique, P. Beng V.P., Kuete, V. 2020. Mechanisms of action of roots crude extract and adianthifolioside GS1 from *Albizia adianthifolia* (Fabaceae) against MDR Gram-negative enteric bacteria. *Investigational Medicinal Chemistry and Pharmacology* **3(2),** 46.
29. Youmbi LM, Atontsa BCK, Tankeo SB, Wamba BEN, Nayim P, Nganou BK, Bitchagno GTM, Simo IK, Mpetga JDS, Penlap VB and Kuete V. 2020. Antibacterial potential and mechanism of action of botanicals and phytochemicals from *Stachytarpheta cayennensis* (Verbenaceae) against Gram-negative multidrug-resistant phenotypes expressing efflux pumps. *Investigational Medicinal Chemistry and Pharmacology* **3(1)**, 35.
30. Park SC, Park Y, Hahm KS. 2011. The role of antimicrobial peptides in preventing multidrug resistant bacterial infections and biofilm formation. *Int J Mol Sci.* 12, 5971-5992
31. Brogden, K. A., M. Ackermann, et al. 2003.Antimicrobial peptides in animals and their role in host defences. *Int J Antimicrob Agents* 22(5), 465-78.
32. Kurtz J, Franz K. 2003. Evidence for memory in invertebrate immunity. Nature 425, 37–38.
33. Rolff J, Siva-Jothy MT. 2003. Invertebrate ecological immunology. Science 301, 472–475.
34. Bergen PJ, Bulman ZP, Saju S, Bulitta JB, Landersdorfer C, Forrest A, Li J, Nation RL, Tsuji BT. 2015. Polymyxin combinations: pharmacokinetics and pharmacodynamics for rationale use. Pharmacotherapy 35,34–42.
35. Guefack, M.G.F., Tankeo, S.B., Ngaffo, C.M.N., Nayim, P., Wamba, B.E.N., Bonsou, I.N., Kuete, V., Mbaveng, A.T. 2021. Antibiotic-potentiation *activities* of three animal species extracts, *Bitis arietans, Helix aspersa,* and *Aristaeomorpha foliacea* and mode of action against MDR Gram-negative bacteria phenotypes. *Investigational Medicinal Chemistry and Pharmacology* 4(1), 48.
36. Kark G, 2018. Ninhydrine test: principle, requirement, procedure, and result. www.onlinebiologynotes. com/ninhidrine-test-principle-requirement-procedure-andresult. Accessed on June 22, 2019.
37. Harbone JB. 1973. Phytochemical methods: A guide to modern techniques of plant analysis. London, Chapman and Hall Ltd. p 116
38. Eloff JN. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med.* 64, 711–713.
39. Tamokou JD, Mbaveng AT, Kuete V. 2017. Antimicrobial activities of African medicinal spices and vegetables. In Kuete, V. eds: Medicinal Spices and Vegetables from Africa, 30: 207-237.
40. Kuete V, Nana F, Ngameni B, Mbaveng AT, Keumedjio F, Ngadjui BT. 2009. Antimicrobial activity of the crude extract, fractions, and compounds from stem bark of *Ficus ovata* (Moraceae). *Journal Ethnopharmacol*. 124, 556–561.

41. Coutinho HD, Vasconcellos A, Freire-Pessoa HL, Gadelha CA, Gadelha TS, AlmeidaFilho GG. 2010. Natural products from the termite Nasutitermes corniger lower aminoglycoside minimum inhibitory concentrations. *Pharmacogn Mag.* 6:1-4.
42. Davies J., Davies D, 2010. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*, 74(3), 417–433
43. Débora L S, Oliveira OP, Cabral MES, Dias DQ, Kerntopf MR, Coutinho HDM, Costa JGM, Freitas FRD, Ferreira FS, Alves RRN, Almeida WO. 2015. Chemical identification and evaluation of the antimicrobial activity of fixed oil extracted from Rhinellajimi, *Pharmaceut Biol.* 53(1), 98–103.
44. Alves RRN, Albuquerque UP. 2013. Animals as a source of drugs: Bioprospecting and biodiversity conservation. In: Alves RRN. Rosa IL, eds. Animals in Traditional Folk Medicine: Implications for Conservation. 1st ed. New York, Dordrecht, London, Berlin Heidelberg: Springer.
45. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. 1999.Phylogenetic perspectives in innate immunity. *Science* 284(5418), 1313-8.
46. Andres E, Dimarcq JL. 2007.[Cationic antimicrobial peptides: from innate immunity study to drug development. Update]." *Medécine et Maladies Infectieuses* 37(4), 194-9.
47. Powers JP, Hancock RE. 2003. The relationship between peptide structure and antibacterial activity. *Peptides* 24(11), 1681-91.
48. Lai Y, Gallo RL. 2009.AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol*. 30(3), 131-41.
49. Lehrer RI, Ganz T. 1999. Antimicrobial peptides in mammalian and insect host defence. *Curr Opin Immunol.* 11(1), 23-7.
50. Hancock RE. 2000. Cationic antimicrobial peptides: towards clinical applications. *Expert Opin Investig Drugs* 9(8), 1723-9.
51. Hancock RE, Sahl HG. 2006.Antimicrobial and host-defense peptides as new antiinfective therapeutic strategies. *Nat Biotechnol.* 24(12), 1551-7.
52. Fritsche TR, Rhomberg PR, Sader HS, Jones RN. 2008. Antimicrobial activity of omiganan pentahydrochloride tested against contempory bacterial pathogens commonly responsible for catheter-associated infections. *J Antimicrob Agents Chemother*. 61, 1092-1098.
53. Harris F, Dennison SR, Phoenix RA. 2009. Anionic antimicrobial peptides from eukaryotic organisms. *Curr Protein Pept Sci.* 10, 585-606.
54. Guinoiseau E. 2010. *[Molécules antibactériennes issues d'huiles essentielles : séparation, identification et mode d'action. Sciences du Vivant [q-bio]]. Thèse de Doctorat, Université de Corse*, France. p 5-99
55. Söderhäll K, Cerenius L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunol. 10, 23–28.
56. Hoffmann JA., Reichhart JM, Hetru C. 1996. Innate immunity in higher insects. *Curr Opin Immunol.* 8, 8–13
57. Gillespie JP, Kanost MR, Trenczek T. 1997. Biological mediators of insect immunity. *Rev. Entomol.* 42, 611–643
58. Frost SDW. 1999. The immune system as an inducible defense. In The ecology and evolution of inducible defenses (ed. R. Tollrian & C. D. Harvell), pp. 104–126. Princeton, NJ: Princeton University Press.
59. Morishima I, Horiba T, Iketani M, Nishioka E, Yamano Y. 1995. Parallel induction of cecropin and lysozyme in larvae of the silkworm, Bombyx mori. *Devl Comp. Immunol.* 19, 357–363.
60. da Silva C, Dunphy GB, Rau ME. 2000. Interaction of Xenorhabdus nematophilus (Enterobacteriaceae) with the antimicrobial defenses of the house cricket, Acheta domesticus. *J Invertebr Pathol.* 76, 285–292
61. Adamo SA. 2004 Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket Gryllus texensis. *J Insect Physiol.* 50, 209–216.
62. Touani FK, Seukep AJ, Djeussi DE, Fankam AG, Noumedem JA, Kuete V. 2014. Antibiotic-potentiation activities of four Cameroonian dietary plants against multidrug-resistant Gram-negative bacteria expressing efflux pumps. *BMC Complement Altern Med.* 14,258.
63. Tankeo SB, Lacmata ST, Noumedem JA, Dzoyem JP, Kuiate JR, Kuete V. 2014. Antibacterial and antibiotic-potentiation activities of some Cameroonian food plants against multi-drug resistant Gram-negative bacteria. *Chin J Integr Med.* 20, 546-554.
64. Tankeo SB, Tane P, Kuete V. 2015. In vitro antibacterial and antibiotic-potentiation activities of the methanol extracts from *Beilschmiedia acuta, Clausena anisata, Newbouldia laevis* and *Polyscias fulva* against multidrug-resistant Gram-negative bacteria. *BMC Complement Altern Med*. 15, 412.
65. Nguenang GS, Mbaveng AT, Fankam AG, Manekeng HT, Nayim P, Wamba BEN, Kuete V. 2018. Tristemma hirtum and five other cameroonian edible plants with weak or no antibacterial effects modulate the activities of antibiotics against gram-negative multidrug-resistant phenotypes. ScientificWorldJournal 2018, 7651482.
66. Ngaffo CMN, Tankeo SB, Guefack MGF, Wamba BEN, Nayim P, Bonsou IN, Kuete V, Mbaveng AT. 2021. *In vitro* antibacterial and antibiotic-potentiation activities of five edible plant extracts and mode of action against several MDR Gram-negative phenotypes. *Investigational Medicinal Chemistry and Pharmacology* 4, 49.
67. Poole K. 2001. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *J Mol Microbiol Biotechnol.* 3, 255-264.
68. Li XZ, Nikaido H. 2004. Efflux-mediated drug resistance in bacteria. *Drugs* 64, 159-204
69. Poole K. 2004. Efflux-mediated multiresistance in Gram-negative bacteria. *Clin Microbiol Infect.* 10, 12-26
70. Lavigne JP. 2007. Effets des antibiotiques et mécanismes de résistance. Bactériologie. P 1-3
71. Adwan G, Mhanna M. 2008. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from specimens. *Middle-East J Sci Res.* 3(3), 134-139.
72. Tchibozo S, Motte-Florac E. 2005. *[Animaux médicaux du benin: Des drogues anciennes toujours actuelles]. Bulletin de liaison de l'Association des Amis du Musée de la Pharmacie* 29, 40-47.
73. Alves RRN, Rosa IL. 2010. Trade of animals used in Brazilian traditional medicine: Trends and implications for conservation. *J Hum Ecol.* 38, 691–704.
74. Alves RRN, Rosa IL, Albuquerque UP, Cunningham AB. 2013. Medicine from the wild: An overview of the use and trade of animal products in traditional medicines. In: Alves RRN, Rosa I, eds. Animalsin Traditional Folk Medicine. Heidelberg, Germany: Springer, 25–42.
75. Alves RRN, Léo Neto N, Santana AGG, Vieira WLS, Almeida WO. 2009. Reptiles used for medicinal and magic religious purposes in Brazil. *Appl Herpetol.* 6, 257–274.
76. Dias DdQ, Cabral MES, Sales DL, Oliveira OP, Filho JAdA, Teles DA, de Sousa JGG, Coutinho HDM, Costa JGM, Kerntopf MR, Alves RRN, Almeida WdO. 2013. Chemical Composition and Validation of the Ethnopharmacological Reported Antimicrobial Activity of the Body Fat of Phrynopsgeoffroanus Used in Traditional Medicine. *Evid Based Complement Alternat Med.* 2013, 715040.
77. Yan F, Zhang YL, Jiang RP et al., 2011.Identifcation and agglutination properties of hemocyanin from the mud crab (Scylla serrata)," *Fish Shellfish Immunol.* 30, 354–360.
78. Yusof WRW, Ahmad FB, Swamy M. 2017. A brief review on the antioxidants and antimicrobial aeptides revealed in mud crabs from the genus of Scylla. *J Mar Biol.* 2017, 1850928.
79. Yeh JY, Huang WJ, Kan SF, Wang PS. 2003. Effects of bufalin and cinobufagin on the proliferation of androgen dependent and independent prostate cancer cells. Prostate 54, 112–124.
80. Nasu K, Nishida M, Ueda T, Takai N, Bing S, Narahara H, Miyakawa I. 2005. Bufalin induces apoptosis and the G0/G1 cell cycle arrest of endometriotic stromal cells: A promising agent for the treatment of endometriosis. *Mol Hum Reprod.* 11, 817–823.
81. Brito SV, Sales DL, Costa JGM, Rodrigues FFG, Ferreira FS, Angélico EC, Carvalho JE, Almeida WO, Anjos LA, Coutinho HDM. 2012. Investigation of the cytocidal potential of Rhinella jimi skin methanol extracts. *Pharmaceut Biol.* 8. 1404-1410.
82. Ziad-Meziane, Hanane-Fadila, Laraba-Djebari F. 2018. *[Purification et identification d'une molécule bactéricide issue de venin de serpent Carastes cerastes].* SAGREN, 2, 23-33
83. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Albert-Franco S, Ngadjui BT, Pagès JM. 2010. Efflux pumps are involved in the defense of Gram-negative bacteria against the natural products Isobavachalcone and diospyrone. *Antimicrob Agents Chemother*. 54, 1749–1752.
84. Lorenzi V, Muselli A, Bernadini AF, Berti L, Pagès J-M. 2009. Geraniol restores antibiotic activities against multidrugs resistant isolates from Gram-negatives species. *Antimicrob Agents Chemother*. 53, 2209-2211.
85. Chevalier J, Pagès J-M, Eyraud A, Malléa M. 2000. Membrane permeability modifications are involved in antibiotic resistance in *Klebsiella pneumoniae*. *Biochemical and Biophysical Resource Commun,* 274, 496-499.
86. Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. 1992. Quinone chemistry and toxicity. *Toxicol Appl Pharmacol.* 112, 2–16.
87. Bagliomi P, Liberatori S, Pallini V, Marri L. 2003. Proteome analysis of Escherichia coli W3110 expressing an heterogenous sigma factor. *Proteomic* 3, 1060-1065.
88. Malléa M, Chevalier J, Bornet C, Eyraud A, Pagès J-M, Davin-Régli A. 1998. Porin alteration and active efflux: two in vivo drug resistance strategies used by Enterobacter aerogenes. *Microbiology* 144, 3003–3009.
89. Ghisalberti D, Masi M, Pagès J-M, Chevalier J. 2005. Chloramphenicol and expression of multidrug efflux pump in *Enterobacter aerogenes*. *Biochem Biophys Res Commun*. 328, 1113-1118.
90. Pradel E, Pagès J-M. 2002. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen *Enterobacter aerogenes*. *Antimicrob Agents Chemother*. 46, 2640-2643.
91. Fredrickson J, Zachara J, Balkwill D. 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford site, Washington State. *Appl Environ Microbiol.* 70, 4230– 4241.